Single-run assay and impurity testing of fixed-dose combination drugs using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution

Technical Overview

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Abstract

The Agilent 1200 Infinity Series High Dynamic Range Diode Array Detection Solution provides a 30× wider linear dynamic UV-range. By combining the signals from two diode array detectors with different path length Max-Light flow cells, the high dynamic range DAD solution detects and quantifies main and trace compounds in a single run without exceeding the linear UV-range.

Using a conventional DAD, typically two injections with different injection volumes are needed to determine the low dose and high dose as well as the trace compounds. At the high injection volumes that are required to determine the low dose and trace compounds, the high dose compounds exceed the linear range of a conventional detector.

This Technical Overview demonstrates that one single injection is adequate to reliably quantify low dose, high dose, and trace compounds of a fixed-dose combination drug using the high dynamic range detection solution from Agilent. The results are compared to those from a conventional DAD. In addition, the precision of areas and limit of detection for both detection solutions are evaluated and compared.

Agilent Technologies

Introduction

Fixed-dose combination drugs are used in the medication of various disease patterns. The composition percentage of the active ingredients can vary depending on the desired physiological effect. If high dose and low dose ingredients are combined, the analysis using conventional HPLC and UHPLC diode array detectors may need at least two injections. This ensures that all compounds are quantified within the linear range of the detector with reliable integration and quantification of trace compounds.

This Technical Overview shows that with the Agilent 1290 Infinity Series High Dynamic Range (HDR) DAD Solution, high dose and low dose compounds as well as their impurities can be determined in a single run with high sensitivity and excellent area precision without exceeding the linear UV-range.

Experimental

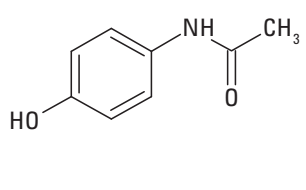
Instrumentation

An Agilent 1290 Infinity LC System consisting of the following modules was used;

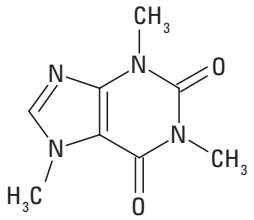
- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Autosampler Thermostat (G1330B)
- Agilent 1290 Infinity Column Compartment (G1316C)
- Two Agilent 1290 Infinity Diode Array Detectors (G4212A)
- Detector cells: 60-mm path length cell (G4212-60007)
  3.7-mm path length cell (G4212-60032)
- Agilent Dide Array 1200 Series Diode Array Detector (G1315C) with 10-mm path length cell

Compounds

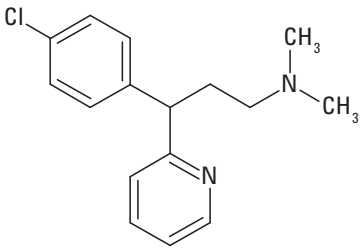
A fixed-dose combination drug was used as sample with the following ingredients: Paracetamol and chlorphenamine with a ratio 1:80, other compounds were vitamin C and caffeine and further small unknown impurities.



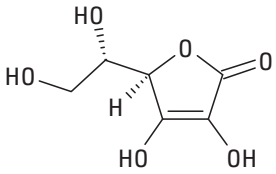
Paracetamol



Caffeine



Chlorphenamine



Vitamin C

Sample preparation

1. Two capsules of a cold medication were opened and dissolved in 20 mL distilled water
2. Extraction with ultrasonic bath for 5 minutes
3. Filtering with Satorious Minisart 0.8-µm filter
4. Clear liquid was filled and stored in 1.5-mL LC vials containing:
   - 250 ng/µL chlorphenamine
   - 20,000 ng/µL paracetamol
   - 2,500 ng/µL caffeine
   - 15,000 ng/µL vitamin C
5. Dilution 1: 20 with water containing:
   - 12.5 ng/µL chlorphenamine
   - 1000 ng/µL paracetamol
   - 125 ng/µL caffeine
   - 750 ng/µL vitamin C
6. This solution was injected

Chromatographic conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902)
Mobile phase:	Water+ 0.1% TFA/Acetonitrile + 0.09% TFA = 95/5
Gradient:	5% B at 0 minutes, 5% B at 0.5 minutes, 40% B at 6.1 minutes, 95% B at 6.5 minutes, 5% B at 8 minutes
Stop time:	10 minutes
Post time:	2 minutes
Injection volume:	1 µL and 5 µL for conventional DAD 5 µL for high dynamic range DAD solution
Column temperature:	40 °C
Detection:	254/20 nm, Ref 380/80, 10 Hz

Software and firmware

Agilent OpenLAB CDS ChemStation revision B.04.03 SP1, C.01.03 and C.01.04 can be used.

All Agilent LC modules had RC.Net drivers with appropriate firmware revision.

Principle of the Agilent 1200 Infinity Series High Dynamic Range DAD Solution

The Agilent 1200 Infinity Series High Dynamic Range DAD Solution provides a 30× wider linear dynamic UV-range. By combining the signals from two diode array detectors with different path length Max-Light flow cells, the high dynamic range detection solution detects and quantifies components with significantly different concentrations in a single run. Two Agilent 1260 or 1290 Infinity DADs are clustered, (Figure 1). One DAD is equipped with a 60-mm path length cell to analyze highly concentrated compounds, and the second DAD is equipped with a 3.7-mm path length cell to analyze low concentrated compounds. The 60-mm cell must be the first in the flow path followed by the 3.7-mm cell. The output signal is a combined signal, normalized to 10-mm path length. The linear UV-range of the 1200 Infinity Series High Dynamic Range DAD Solution is typically as wide as 0.6×10^{-6} to 6.7 AU/cm. In comparison, the earlier Agilent 1200 Series DAD has a maximum linear range of 7×10^{-6} to 2 AU/cm.

The high dynamic range (HDR) tool can be activated through the ChemStation by selecting the appropriate field, (Figure 2). For adjustment of the retention time shift between the detector cells, the delay volume must be entered. This is the volume of the connection capillary between the 60-mm and 3.7-mm path length cells. A capillary with a volume of 0.0125 µL is supplied with the 3.7-mm path length cell.

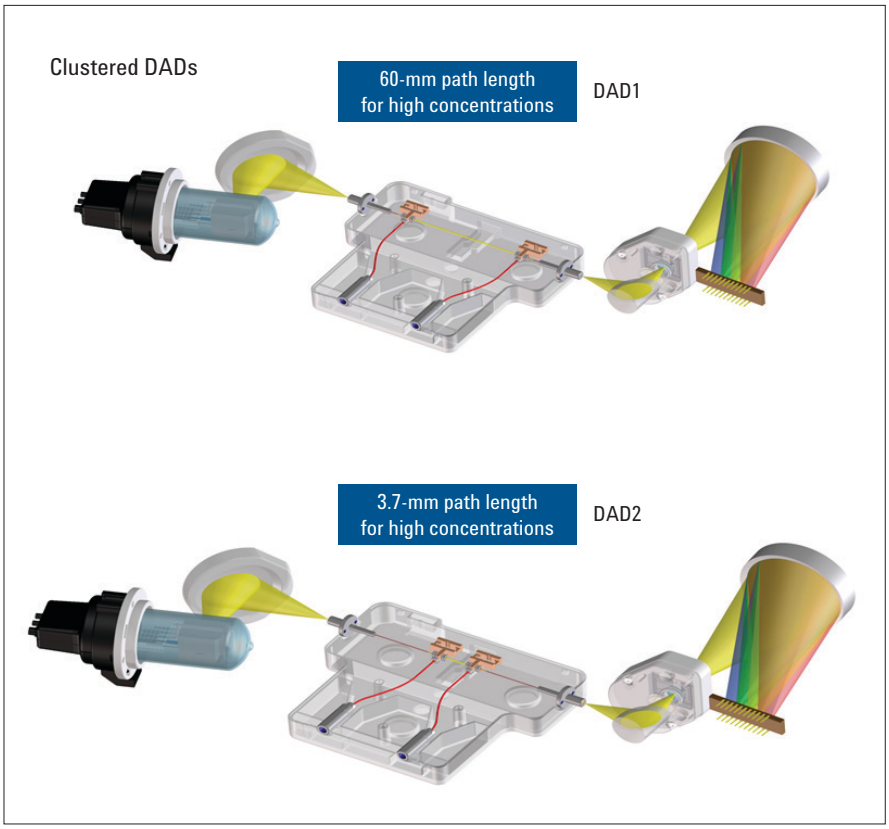


Figure 1
Clustered 1200 Infinity Series DADs.

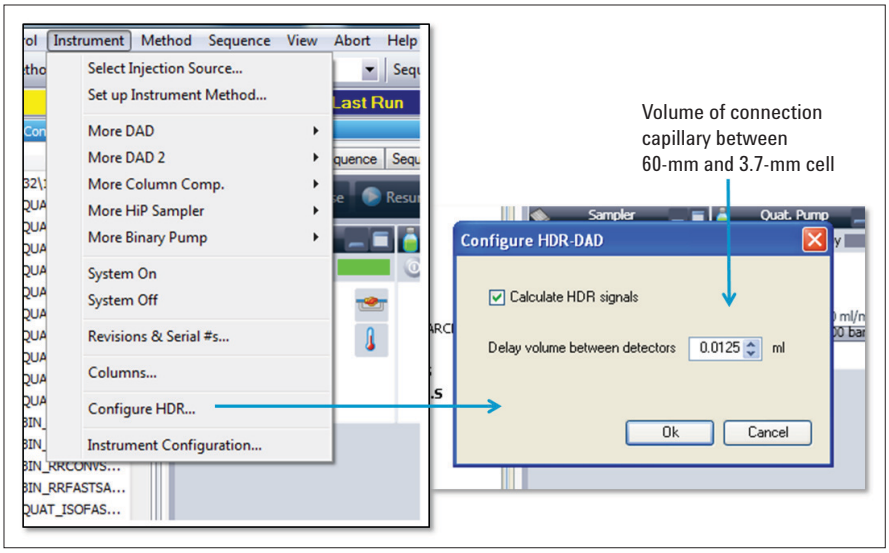


Figure 2
Activating the HDR tool in the ChemStation software.

Results and discussion

For all experiments the same Agilent 1290 Infinity LC System configuration was used with the exception of the detectors. To evaluate the performance differences between the conventional DAD and the Agilent high dynamic range solution, the following experiments were performed:

- Analysis of a fixed-dose combination drug on a 1290 Infinity LC System with conventional DAD using two injections of 1 and 5 µL volumes to determine high and low-dose drugs and further trace compounds.
- Analysis of a fixed-dose combination drug on a 1290 Infinity LC System with the HDR solution using one single injection of 5 µL volume to determine low-dose and high-dose drugs, and further trace compounds within the linear UV-range of typically 6.7 AU.

For the first experiment, the conventional DAD was used. Two injections were necessary to determine all compounds and trace impurities within the linear UV-range, (Figures 3, 4). Vitamin C, paracetamol, and caffeine were determined by injecting 1 µL. The maximum peak height was approximately 1,500 mAU, which is within the linear UV-range of the conventional DAD. The other compounds were determined by injecting 5 µL. Vitamin C and paracetamol were now at a maximum peak height of 3,800 mAU out of the UV-linear range.

Figure 4 shows enlarged the 1- and 5-µL injections using the conventional DAD. Only with the 5-µL injection it was possible to reliably identify and determine chlorphenamine and further trace compounds. For chlorphenamine the peak height for the 1-µL injection was only as low as 3.7 mAU, whereas for the 5-µL injection approxiamtely 22 mAU were achieved. This ensured easier integration and subsequently more reliable quantification. The impurities 3 and 4 (impurities 1 and 2 elute earlier in the chromatogram, Figure 6) could only be analyzed by injecting 5 µL.

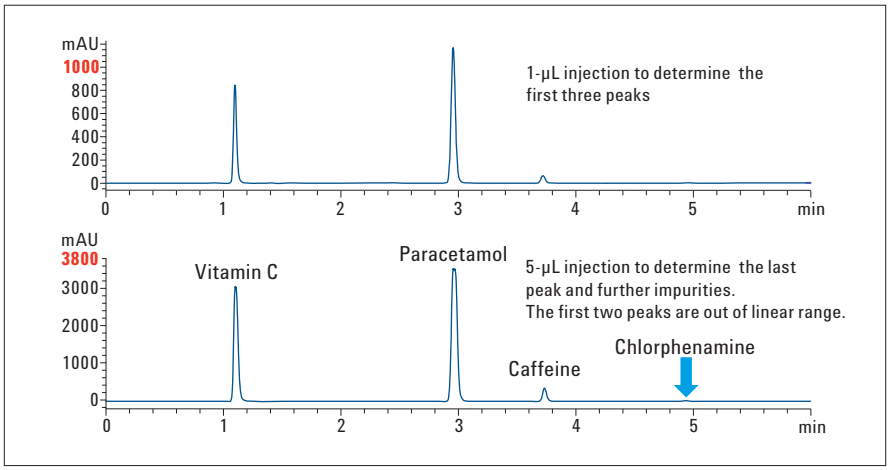


Figure 3
Chromatograms of the drug using two injections with different injection volumes.

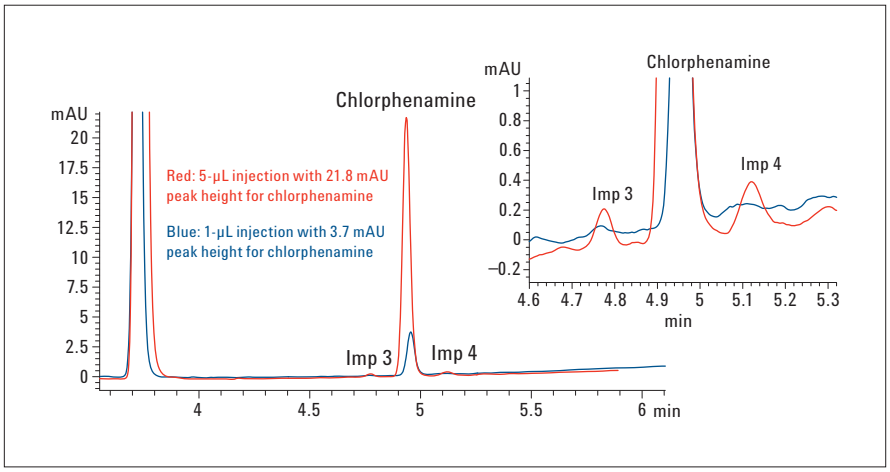


Figure 4
Enlargement of the 1-µL and 5-µL injections for the conventional DAD.

In a second experiment, 5 µL of the fixed-dose combination drug was injected using the 1290 Infinity HDR DAD Solution. Figure 5 shows an overlay of the 5-µL injection of the conventional DAD signal and the HDR DAD signal. Using the HDR DAD signal, all peaks were within the linear range and could be precisely quantified. The HDR DAD signal is close to 4,000 mAU, which is within the linear UV-range for the HDR DAD of approximately 6,000 mAU. Consequently vitamin C, paracetamol and caffeine could be quantified without problems by saturated signals.

Figure 6 shows enlargement of the 5-µL injection for the conventional and the HDR DAD signal.

As expected, the HDR DAD signal allowed the evaluation of chlorphenamine and impurities 1-4 simultaneously with the determination of the high-dose compounds as shown in Figure 5

Performance evaluation

The limit of detection with S/N = 3 of low-dose compound chlorphenamine was evaluated for the conventional DAD and the HDR DAD solution, (Table 1). The limit of detection for the HDR DAD signal was 10 times lower than for the conventional DAD signal. This is mainly due to the excellent noise performance of the HDR DAD signal, (Figure 7). For the limit of detection calculation the noise range between 4.2 and 4.6 minutes was chosen.

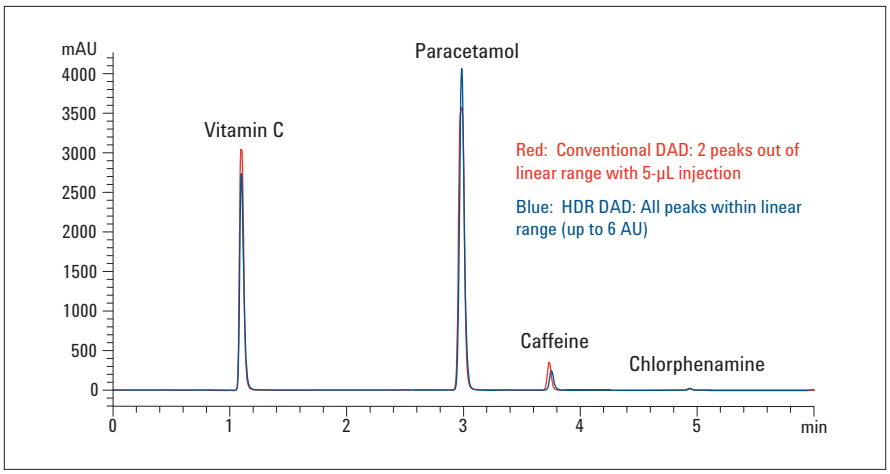


Figure 5
Overlay of 5 µL injection of conventional DAD signal and HDR DAD signals.

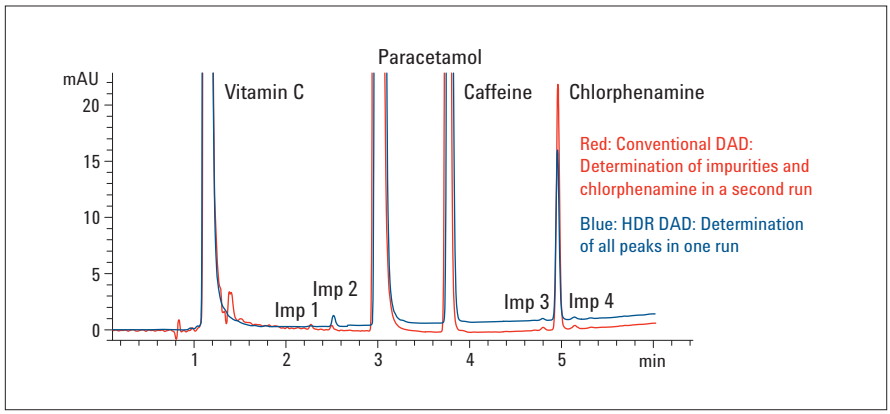


Figure 6
Enlargement of 5-µL injection of the conventional and the HDR DAD signals.

	LOD with S/N=3 for conventional DAD	LOD with S/N=3 for HDR DAD
Chlorphenamine	~1 ng	~ 0.1 ng

Table 1
Limit of detection chlorphenamine for the conventional DAD and the HDR DAD signals.

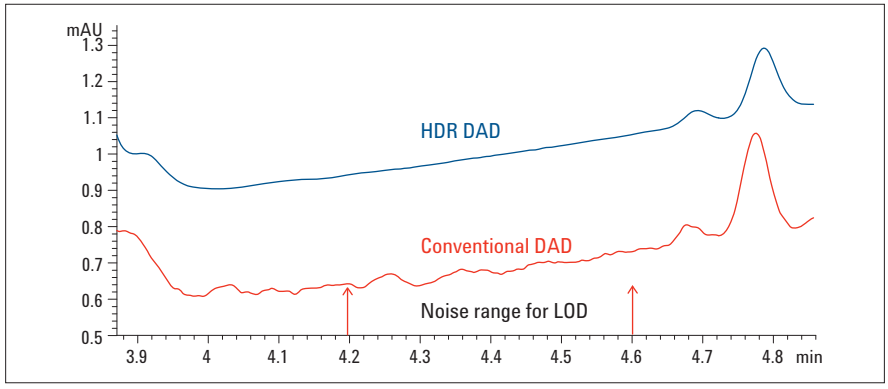


Figure 7
Baseline noise of conventional DAD and HDR DAD signals.

The area precision was evaluated for all main and trace compounds, (Figure 8). The blue bars represent the HDR DAD signal. The red bars represent the 1-µL injection of the conventional DAD that was used to determine vitamin C, paracetamol, and caffeine. The green bars represent the 5-µL injection of the conventional DAD that was used to determine chlorphenamine and impurities 1 to 4.

The HDR DAD signal provided significantly better precision for all compounds. For vitamin C, paracetamol, caffeine, and chlorphenamine the area precision was < 0.1% RSD. The area precision for the impurities was between 1 and 3% RSD.

For the conventional DAD, the area precision for vitamin C, paracetamol and caffeine and chlorphenamine was < 2% RSD and for the impurities between 5 and 15% RSD.

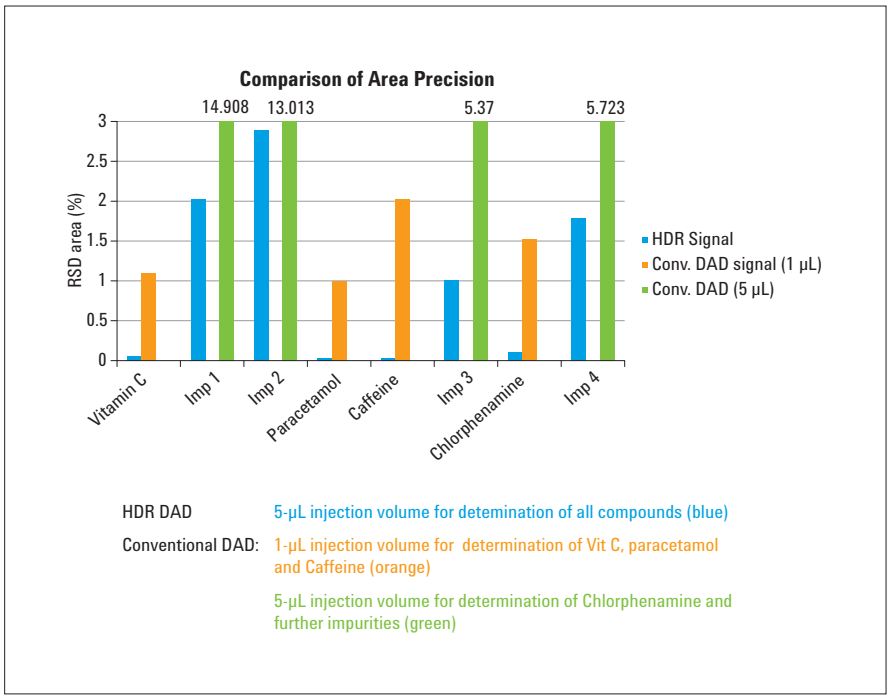


Figure 8
RSD of areas for conventional DAD and HDR DAD signals.

Conclusion

With the Agilent 1290 Infinity HDR DAD Solution, determination of low and high-dose drugs and additional impurities present in the analyzed fixed-dose combination drug was possible in one run. The complete determination of all compounds using the conventional DAD required two injections with different injection volumes to avoid saturated signals for the high-dose drugs.

Further, significantly improved precision and lower detection limits were achieved with the 1290 Infinity HDR DAD Solution compared to the conventional DAD. The limit of detection for chlorphenamine was 10 times better using the HDR DAD solution. The area precision for the additional unknown impurities was three to seven times better. For the high dosed ingredient paracetamol, the area precision was 30 times better for the HDR DAD signal.

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